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May 19, 2005

Herbert A. Smith, Ph.D.
Division of Dockets Management (HFA-305)
Food and Drug Administration
5630 Fishers Lane, Rm. 1061
Rockville, MD 20852

Re: Docket No. 2005D-0047

Dear Dr. Smith:

Vical Incorporated hereby submits comments to the draft Guidance for Industry "Considerations for Plasmid DNA Vaccines for Infectious Disease Indications". We have included the entire text of the draft guidance document for reference. Our comments follow each paragraph in bold and italic text. Please feel free to contact me at (858) 646-1117 or by email if you have any questions.

Regards,

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Guidance for Industry
Considerations for Plasmid DNA Vaccines for Infectious Disease Indications
Contains Nonbinding Recommendations

Draft - Not for Implementation

This draft guidance, when finalized, will represent the Food and Drug Administration's (FDA's) current thinking on this topic. It does not create or confer any rights for or on any person and does not operate to bind FDA or the public. You can use an alternative approach if the approach satisfies the requirements of the applicable statutes and regulations. If you want to discuss an alternative approach, contact the appropriate FDA staff. If you cannot identify the appropriate FDA staff, call the appropriate number listed on the title page of this guidance.

I. INTRODUCTION

In December 1996, FDA issued a guidance document, "Points to Consider on Plasmid DNA Vaccines for Preventive Infectious Disease Indications," to assist the developers of DNA vaccines. That document delineated the manufacturing, preclinical, and clinical issues relevant to the development of DNA vaccines, and described potential safety concerns that we, FDA's Center for Biologics Evaluation and Research (CBER), recommended vaccine developers address prior to the initiation of phase 1 clinical studies. The recommendations for DNA vaccine manufacture and testing provided in that document were based on our experiences with other types of vaccines and DNA-based products, including gene therapy agents.

In the intervening years, we have concurred with the initiation of phase 1 clinical studies of DNA vaccines for a number of infectious disease indications including malaria, hepatitis B, and human immunodeficiency virus (HIV). The initiation of phase 1 clinical studies is predicated on you, the manufacturers and/or sponsors of vaccine clinical studies, documenting the quality and consistency of plasmid manufacture, combined with extensive preclinical safety studies. Considerable clinical experience has been accumulated since the issuance of the above 1996 guidance on plasmid DNA vaccines, and we need to update that guidance. This guidance, when finalized, will update and replace the 1996 guidance document.

FDA helps ensure that clinical studies provide critical information on vaccine safety and immunogenicity without placing undue or unreasonable demands on vaccine study sponsors. Ongoing interactions between FDA and vaccine study sponsors are designed to achieve these goals. This update to the 1996 Points to Consider document describes our current recommendations for the development and testing of DNA vaccines.

For the purposes of this document, DNA vaccines are defined as purified preparations of plasmid DNA designed to contain one or more genes from a pathogen as well as regulatory genetic elements to enable production in a bacterial host system. Typically, these plasmids possess DNA sequences necessary for selection and replication in bacteria. In addition, they contain eukaryotic promoters and enhancers as well as transcription termination/polyadenylation sequences to promote gene expression in vaccine recipients, and may contain immunomodulatory elements. DNA vaccines are biological products as set forth in section 351 of the Public Health Service Act (PHS) (42 U.S.C. 262) and are regulated by CBER. The principal regulations applicable to DNA vaccines are located in 21 CFR Parts 210, 211, 600, 601, and 610. Other guidance documents are available from CBER and may contain information that is relevant to DNA vaccines. Some of these documents are listed below and additional guidance documents may be found on the CBER website (http://www.fda.gov/cber/guidelines.htm) or the CDER website (http://www.fda.gov/cder/guidance/index.htm).

This document is intended to assist you in your development of DNA vaccines to prevent infectious diseases. This guidance is not necessarily applicable to DNA vaccines for the treatment of established diseases (infectious or malignant), since subjects with ongoing disease may require more aggressive therapy with a different margin of safety than prophylactic vaccines administered to healthy individuals. Applications for DNA vaccines designed to prevent or treat infectious diseases should be submitted to CBER's Office of Vaccines Research and Review (OVRR) where primary review responsibility is assigned. Plasmid DNA products intended for non-infectious therapeutic indications are not addressed in this guidance. Applications for these products should be submitted to CBER's Office of Cellular, Tissue and Gene Therapies (OCTGT).

FDA's guidance documents, including this guidance, do not establish legally enforceable responsibilities. Instead, guidances describe FDA's current thinking on a topic and should be viewed only as recommendations, unless specific regulatory or statutory requirements are cited. The use of the word should in FDA's guidances means that something is suggested or recommended, but not required.

II. MANUFACTURING ISSUES

The following sections describe the manufacturing information we recommend that you submit to us for a new DNA vaccine product for clinical study under an Investigational New Drug Application (IND).

A. Product Manufacture

We recommend that the manufacturing summary describe all components used during manufacture as well as those present in the final product. We recommend that you provide detailed descriptions of the plasmid construction, including the source and diagrams of all plasmids used, and all intermediate recombinant DNA cloning

procedures. We recommend that the DNA sequence of the entire plasmid be provided by direct sequencing of the plasmid present in the Master Cell Bank (MCB). During production, other methods of sequence verification, such as restriction enzyme mapping and polymerase chain reaction (PCR) may be employed at intermediate steps.

Comment:

> ***If a complete, annotated nucleotide sequence of the plasmid in the MCB is provided in the initial phases of product development, a detailed description of the methods and sources used to construct the plasmid does not seem necessary to evaluate the safety of the plasmid. Please include additional reasons that justify reviewing the methods and sources of plasmid construction for an initial IND if the complete, annotated nucleotide sequence is provided.***

We recommend that you describe the genotype, phenotype, source of the bacterial cells and the procedures to construct master and working cell banks used for production. Specific guidance for the establishment of MCBs and Working Cell Banks (WCBs) is described in the "Points to Consider in the Characterization of Cell Lines Used to Produce Biologicals (1993)".[1] We recommend that you test both the MCBs and WCBs to ensure that they are free from bacteriophage and other adventitious agent contamination, and that you establish the genetic stability of the MCB and WCB.

Comment:

> ***The requirement that we establish the genetic stability of the MCB and WCB used for the manufacture of Phase 1 Clinical Trial Material needs clarification. Genetic stability could refer to the stability of the host cell genome and/or the stability of the plasmid DNA. We assume the emphasis is on the stability of the plasmid DNA because we do not believe there is a need to establish genetic stability of the host cell genome in the absence of evidence to suggest host cell genome instability.***
>
> ***We suggest that the genetic stability analysis be limited to only one cell bank and testing be performed one time because:***
> ***(1) some manufacturers may use only an MCB to manufacture material during early product development, and therefore, are limited to analyzing only one cell bank; and***
> ***(2) if a manufacturer uses a WCB derived from the MCB, then by establishing genetic stability of the WCB, the genetic stability of the MCB (the parent cell bank) is reasonably assumed.***
>
> ***With regard to the type of analysis required to establish genetic stability, there are two general approaches. One approach is the establishment of genetic stability at a polynucleotide structural level and the other is to establish genetic stability at the single nucleotide level. For example, polynucleotide structural genetic stability of plasmid DNA is confirmed by restriction enzyme mapping of***

the plasmid DNA isolated from the MCB. These assays are used routinely to release each bulk plasmid DNA drug substance as tests for identity and purity (the presence or absence of unwanted structural mutants, such as large deletions, substitutions or rearrangements). In contrast, the establishment of genetic stability of the plasmid DNA at the nucleotide level requires that:
(1) a statistically appropriate number of overlapping sequences be generated by direct sequencing of the same plasmid DNA sample, and;
(2) sequences be generated from plasmid DNA isolated from a statistically appropriate number of different plasmid preparations to detect single nucleotide changes at the specified frequency and confidence level (e.g., given that the true occurrence rate of a mutation at a specific nucleotide location in the plasmid population being sampled is 10%, there is a 95% confidence of detecting at least one occurrence of a mutation at a specific nucleotide location if 29 independent plasmid preparations are analyzed; likewise, given a true occurrence rate of 1%, there is 99% confidence in detecting at least one occurrence of a mutation at a specific nucleotide location if 459 independent plasmid preparations are analyzed).
This latter approach seems excessive for Phase 1 Clinical Trial Material, particularly if the DNA sequence of the entire plasmid has been provided by direct sequencing of the plasmid present in the MCB. Therefore, we recommend for the early development phases of the product that the genetic stability analysis be limited to:
(1) the plasmid DNA and not the bacterial genome;
(2) only one cell bank (either MCB, if there is no WCB; or WCB, if one is available);
(3) a polynucleotide structural level analysis rather than a nucleotide level analysis.

We recommend that the description of the manufacturing process be sufficiently detailed to enable an assessment of the safety of the product. If changes in product manufacture occur during the development of plasmid products intended for clinical studies and preclinical lots manufactured for safety evaluation, we recommend that you provide a clear summary illustrating all differences between lots of vaccine used in preclinical studies and those intended for use in clinical studies.

B. Bulk Plasmid Product Release Testing

If bulk and final product are the same (i.e., if production runs yield one lot and no further steps in formulation are performed), then testing as described below may be redundant and unnecessary. We recommend that you test bulk plasmid products for the properties described below, and that you use standard assay(s) of adequate specificity and sensitivity. We recommend that you evaluate assay methods by testing known amounts of reference materials or spiked samples, or by other appropriate measures, and that you submit to CBER data documenting assay performance. In addition to bulk and final

product release testing, we recommend that you also perform in-process testing to ensure manufacturing consistency and product safety. Prior to the initiation of phase 1 clinical studies, we recommend that you initiate stability testing as early as possible to support use of the product for the duration of the proposed clinical investigation.

Comment:

> ***In the early stages of product development, assays are usually developed using a research standard. A brief description of these assays and the final product lot release data in a Phase 1 IND submission should be sufficient information to evaluate a manufacturer's ability to execute the assays.***

Typically, the bulk release criteria will include tests for visual appearance and plasmid concentration. We recommend that the bulk release criteria describe the extent of circular plasmid present with establishment of a minimum specification. We recommend that you characterize the product for the extent of supercoiled plasmid in the bulk preparation and that you establish a minimum specification (preferably >80%). We recommend that you evaluate bulk plasmid preparations for the presence of bacterial host cell contaminants to include DNA, RNA, and protein and set limits for the maximum level of each of these contaminants. We generally recommend that host cell contaminants be at as low a concentration as is technically feasible. We recommend that you perform a test for pyrogenic substances and that you include the test results with the bulk release documentation. The Limulus Amebocyte Lysate (LAL) test is a sensitive indicator of the presence of bacterial endotoxins and endotoxin contamination should not exceed 5.0 EU/kg body weight for the intended recipients.

Comment:

> ***The percent supercoiled specification for bulk drug substance (containing only one plasmid) is measured to monitor manufacturing consistency. A justification for the specification of >80% supercoiled (SC) for all plasmids should be provided in the guideline. Data in a recent publication (Cupillard et. al. <u>Vaccine</u> 23: 1910-1916, 2005) suggests 48% SC works just as well as 81% in a rabies model. An arbitrary specification for manufacturing could inhibit the development of manufacturing technology. If the agency has supportive data for the proposed level of 80%, either such information should be provided or the level should be supported by a rationale.***
>
> ***We agree that a lower limit of host cell macromolecules should be evaluated for Phase 1 material. However, we recommend that limits be set based upon assay detection limits and previous experience with plasmid DNA products. We believe the guidance should state that "during Phase 3 manufacturing and process validation, limits can be set based upon the technical feasibility of the manufacturing process and safety data" instead of "as low a concentration as technically feasible". We also suggest that the word "contaminants" be changed to "macromolecules."***

Are the bulk tests described in this section intended for material that contains only a single plasmid? If so, what tests would be necessary for an individual plasmid that will be mixed with one or more plasmids to form a bulk mixture?

We recommend that you include a test to establish the identity of the bulk product by restriction enzyme analysis in the bulk release criteria. When a single manufacturing facility is used to manufacture more than one DNA vaccine product, we recommend that you perform identity tests capable of distinguishing individual plasmids.

Comment:

We believe an example of an identity test should be added to the above paragraph. For example, "Agarose gel electrophoresis of plasmid DNA after restriction enzyme digestion is one test that can be used to identify and distinguish individual plasmids."

We recommend that you develop a potency assay. During early product development, we will allow sponsors considerable flexibility in the selection of potency assays. This could include in vitro measures of transfection efficiency that monitor the transcription and/or translation of the encoded gene(s). Assays that monitor in vivo immunogenicity of the DNA vaccine are preferred. We recommend that assays be quantitative. We recommend that as product development proceeds towards licensure, you provide evidence that in vitro potency correlates with in vivo immunogenicity. We recommend that sponsors maintain retention samples of each lot to facilitate comparisons between lots as assay development progresses. The selection and implementation of a potency assay may be discussed with CBER to ensure acceptability of the design.

Comment:

We suggest that a statement acknowledging the limitations of correlating in vitro potency and in vivo immunogenicity data generated from assays that normally have inherently variable results, be included in this section. Also, include any thoughts the agency has on developing potency assays based upon laboratory animal studies and the possibility that animal immunogenicity might not correlate to human immunogenicity.

C. Final Product Release Testing

We recommend that you test the final DNA vaccine product for potency, general safety, sterility, purity, quantity, and identity. The test methods and specifications may be the same as those employed for the bulk product release. To detect extraneous toxic contaminants potentially introduced during manufacture, we recommend that you perform the general safety test in mice and guinea pigs on each final product lot. If the plasmid product is lyophilized we recommend that you perform a test for residual moisture. We recommend that you perform a test for the presence of endotoxin on each

lot of final product. In addition to final product release testing, we recommend that you also perform in-process testing to ensure manufacturing consistency and product safety. We recommend that you establish acceptance criteria and acceptable limits and that you report the results for each lot of vaccine to be used for clinical studies.

Comment:

> ***It should be restated here that certain bulk release tests do not have to be repeated on the final product if there is no change in the final composition. "In-process testing" for the final product is assumed to be different from in-process testing carried out during bulk manufacturing. If this assumption is correct, the intent should be stated that the referral is to environmental monitoring and other tests associated with aseptic processing.***
>
> ***For a Phase 1 manufacturing process, if the sponsor uses the same lot for human studies as the one used for preclinical GLP toxicology testing, general safety testing is redundant.***
>
> ***During early process development, the analytical tests intended to monitor and measure manufacturing capability will not have "acceptable limits" established or acceptance criteria until a significant number of lots are manufactured. Therefore, analytical assays intended to measure manufacturing consistency should be reported without acceptance criteria and limits for Phase 1 and 2 material. During Phase 3, manufacturing and process validation acceptance criteria and limits would be determined. We agree that setting limits for Phase 1 analytical assays that measure product safety that are product independent (endotoxin assays, sterility tests, and bioburden in-process tests, etc.) is reasonable.***
>
> ***A statement should be added to address final product formulations with ingredients that interfere with the LAL endotoxin test.***

III. DNA VACCINE MODIFICATIONS

A. Changes to the Insert or Vector

Changes to the DNA sequence of the insert gene or vector sequences of a DNA vaccine would require the submission of a new IND (See section 351 of the PHS Act and 21 CFR Part 312). We recommend that you include in the IND a description of the manufacturing process and the results from preclinical safety evaluation of the new (modified) DNA vaccine.

Comment:

> ***The extent of preclinical safety studies should be measured against the nature of sequence changes and the clinical experience of using those sequences in previous human clinical trials. In circumstances where one or two base-pair changes are made to the plasmid in regions not expressed in eukaryotic cells (vector backbone sequences), a new IND or additional preclinical safety studies should not be required automatically.***
>
> ***As the technology develops, certain clinical studies may be designed to compare expression of genes under the control of different promoters. These projects would be best managed by both industry and FDA under one IND rather than under a separate IND for each plasmid DNA construct.***

B. DNA Sequence Analysis

An issue of product identity of particular relevance to DNA vaccines concerns the degree to which plasmids should be sequenced before the initiation of phase 1 clinical studies. In 1996, we recommended that manufacturers provide (at a minimum) the sequence of the protein-encoding gene insert. Based on evidence that the plasmid backbone may influence vaccine activity, and recognizing that technological advances since 1996 have facilitated DNA sequencing, we recommend that manufacturers provide the complete sequence of the plasmid before initiating phase 1 clinical studies.

Comment:

> ***We recommend a reference be provided to support the statement that the plasmid DNA backbone influences vaccine activity. In addition, we recommend that some sense of the magnitude of the change in the plasmid backbone that appears to be required to have a biologically significant effect on vaccine activity be included in the text above.***

Some DNA vaccines contain a complex mixture of plasmids, with each plasmid carrying a gene encoding a different antigenic protein. For example, a vaccine may contain multiple variants of a highly mutable gene (such as the gene encoding the envelope of HIV-1) or the entire genome of a microorganism may be 'shotgun cloned' into a common plasmid backbone. We advise you to establish the identity and amount of each plasmid component in the vaccine preparation to ensure lot-to-lot consistency. However, there may be instances when technical limitations prevent complete sequence information from being obtained on a heterogeneous mixture of plasmids before initiation of phase 1 clinical studies. In such instances, the amount of sequence information required will be evaluated on a case-by-case basis.

Comment:

We suggest that complex plasmid DNA mixtures formulated using individual bulk plasmid DNA drug substances manufactured from independent master cell banks be excluded from the concepts presented in the above paragraph.

IV. PRECLINICAL IMMUNOGENICITY AND SAFETY

A. General Considerations

Preclinical safety evaluation is required for all new vaccines, including DNA vaccines, prior to their use in clinical studies (21 Code of Federal Regulations (CFR) 312.23). We recommend that you perform preclinical safety studies on every novel DNA vaccine or DNA vaccine/adjuvant combination. We may modify the preclinical safety evaluation requirements in specific situations where multiple variants of a specific gene (such as HIV-1 Env) are cloned into the same plasmid vector on which a complete safety evaluation has already been performed. We recommend that you consult with CBER well in advance of IND submission to evaluate the adequacy of preclinical safety studies and prior human experience to support the investigational vaccine product. Pivotal animal safety studies must be performed in accordance with Good Laboratory Practice (GLP) regulations (21 CFR Part 58).

Comment:

In addition to stating that the FDA "may modify the preclinical safety evaluation requirements in specific situations where multiple variants of a specific gene are cloned into the same plasmid vector," we suggest the following statement be added, "After preclinical safety testing, combining different parts of the plasmid backbone vector (non-antigen coding region) with a different antigen coding region that has also undergone preclinical safety testing will be considered for a modified preclinical safety evaluation".

Plasmid vaccines using Vical's backbone formulated with in lipid or poloxamer-based delivery systems and in phosphate buffer have undergone preclinical safety testing. No safety concerns related to these formulations or plasmid backbone have been identified. In our studies with cationic lipid and poloxamer formulations, formulation-dependent effects on biodistribution have not been detected nor have we observed any evidence that formulated plasmid DNA integrates into the host genomic DNA. (See references listed). However, there may be an impact on persistence of plasmid copy number at the site of injection. It should be sufficient to test the safety of a particular formulation using only one plasmid DNA. An IND using that same formulation with a different plasmid DNA administered by the same route should be allowable without repeating the biodistribution/integration studies.

It is not clear what is meant by pivotal animal safety studies. We recommend that the term "animal toxicology safety studies" be used when referring to GLP studies, for example, a repeat-dose toxicology study.

We recommend that certain animal studies be accepted for clinical trial support as long as sufficient documentation is maintained to recreate the events, even though non-critical parts of 21 CFR 58 are not completely met. We believe this approach is consistent with relevant regulations and guidelines governing toxicology versus pharmacology studies. For example, biodistribution and integration studies should be considered pharmacology studies and not toxicology studies.

References:

Adrián Vilalta, Rohit K. Mahajan, Jukka Hartikka, Denis Rusalov, Terrie Martin, Vesselina Bozoukova, Vicky Leamy, Keith Hall, Peggy Lalor, Alain Rolland, David C. Kaslow . Part I. Poloxamer-formulated plasmid DNA-based human CMV vaccine: Evaluation of pDNA biodistribution/persistence and integration. (Manuscript Submitted for Publication, 2005)

Adrián Vilalta, Rohit K. Mahajan, Jukka Hartikka, Denis Rusalov, Terrie Martin, Vesselina Bozoukova, Vicky Leamy, Keith Hall, Peggy Lalor, Alain Rolland, David C. Kaslow. Part II. Cationic Lipid- formulated plasmid DNA-based Bacillus Anthracis **vaccine:** *Evaluation of pDNA persistence and integration potential. (Manuscript Submitted for Publication, 2005)*

B. Immunogenicity

We recommend that you develop assays to assess immunological potency in animal models. This could include the evaluation of antigen-specific antibody titers, seroconversion rates, activation of cytokine secreting cells, and/or measures of cell-mediated immune responses. Optimally, these studies are designed to collect information regarding the duration of the immune response. For complex DNA vaccines encoding multiple antigens, we recommend that you assess the immune response generated against a representative subset of the encoded antigens.

Comment:

It is not clear if evidence of animal immunogenicity is a requirement for Phase 1 or for further product development. Our understanding is that animal immunogenicity does not always correlate to human immunogenicity. Therefore, a statement justifying the development of this information is necessary, especially for duration of the immune response. We suggest that the guideline state that the development of the immunological potency assay in an animal model should be undertaken only if a relevant animal model exists for the application under study. The definition of "complex DNA vaccines" is

necessary to understand the impact of the requirement to test subsets of encoded antigens.

C. Autoimmunity

Published preclinical studies indicate that DNA vaccination can activate autoreactive B cells to secrete IgG anti-DNA autoantibodies (See Section VI, References). However, the magnitude and duration of this response appears to be insufficient to cause disease in normal animals or accelerate disease in autoimmune-prone mice. These preclinical studies helped to establish that systemic autoimmunity is unlikely to result from DNA vaccination. Similarly, the absence of an immune response against cells expressing the vaccine-encoded antigen (including muscle cells and dendritic cells) suggests that an autoimmune response directed against tissues in which such cells reside is unlikely. Based on these findings, we will no longer expect that you perform preclinical studies to specifically assess whether vaccination causes autoimmune disease.

The possibility persists that DNA vaccines might idiosyncratically cause or worsen organ-specific autoimmunity by encoding antigens (including cryptic antigens) that cross-react with self. Thus, we recommend that you continue to monitor the general well being of animals participating in preclinical immunogenicity and toxicity studies, and of all human trial participants. In cases of immunity developing against a transgene product (such as a cytokine), we recommend that you examine potential cross-reactivity with the corresponding endogenous protein. Studying an animal model using a construct containing the analogous animal gene is recommended to evaluate potential adverse effects.

D. Tolerance

Published studies to address whether DNA vaccines could induce neonatal tolerance yielded divergent results (see Section VI, References). Most DNA vaccines did not induce tolerance in neonatal animals, but idiosyncratic examples of neonatal tolerance have been observed (see Section VI, References). Tolerance has never been observed following vaccination of mature animals. Taken together, these studies suggest that the capacity of a DNA vaccine to induce tolerance may depend on the nature of the encoded antigen and the age at which, and frequency with which, the vaccine is administered. Based on these findings and other considerations, we recommend that prior to use of a DNA vaccine in children or newborns that: i) you first test the vaccine for safety and immunogenicity in adults, and ii) you utilize appropriate preclinical models to evaluate the potential of such vaccines to induce neonatal tolerance.

E. Challenge/Protection, Cytokines, Prime/Boost

When appropriate and where possible, we encourage animal challenge/protection studies with the corresponding infectious agent early in development to demonstrate the rationale

for the use of the investigational vaccine. For DNA vaccines that co-express cytokine genes, you should consider specific preclinical studies in animal species responsive to the encoded human cytokine or models using the analogous animal genes to assess whether modulation of the cellular or humoral components of the immune system might result in unintended adverse consequences, such as generalized immunosuppression, chronic inflammation, autoimmunity or other immunopathology. When plasmid DNA vaccines are used in vaccination strategies employing a corresponding subunit vaccine, such as in prime and boost study designs, we recommend that you submit specific preclinical information to support the safety and tolerability of the proposed dose, schedule, and route of administration of each vaccine combination.

Comment:

> ***Because relatively few human pathogens have animal models acceptable for studies of human cytokines, emphasis should be made that these studies are not always possible as stated for animal challenge protection studies.***

F. Local Reactogenicity and Systemic Toxicity Studies

Studies designed to assess systemic toxicity may be combined with assessment of local site reactogenicity. We recommend that you conduct these studies using the highest dose of vaccine planned for clinical use. You may conduct studies of additional doses to provide further support for vaccine safety. An accelerated schedule of vaccine delivery will be considered (preferably vaccination intervals of 3 to 4 weeks), and should include at least one immunization beyond that planned for clinical use. We recommend that the assessments written into the preclinical study protocols include toxicity to potential target organs, including the hematopoietic and immune systems. We recommend that preclinical studies also include clinical pathology assessments (serum chemistry, hematology, and coagulation tests), and histopathology, encompassing both gross and microscopic assessment of tissues.

Comment:

> ***Evaluation of the highest dose of vaccine planned for human clinical use is not always possible in animals due to the volume of the drug product relative to the injection site of the animal.***
>
> ***An accelerated schedule of vaccine delivery is acceptable for systemic toxicity studies, but the preference for intervals of 3 to 4 weeks may not always reflect what is planned for use in humans; in some indications, humans may be administered vaccine every 1 or 2 weeks.***
>
> ***Immunogenicity data derived from protein-based rather than gene-based vaccines may not be applicable; therefore, reference(s) to immunogenicity data and/or the theory for the preference of 3-4 weeks as an accelerated schedule for DNA vaccine delivery should be provided.***

We recommend that studies of local site reactogenicity include detailed clinical observations of the injection site(s) following each vaccine administration and histological evaluations of injection-site tissue obtained from biopsies or term necropsy samples. We recommend that you evaluate both short-term and persistent toxicity, preferably by studying separate cohorts of animals 2 to 3 days and 2 to 3 weeks after the final vaccination.

G. Biodistribution and Integration Analysis

Plasmid biodistribution, persistence and integration studies were initially recommended by CBER to determine whether subjects in DNA vaccine trials were at heightened risk from i) the long-term expression of the encoded antigen either at the site of injection or an ectopic site, and/or ii) integration of the plasmid that might increase susceptibility to malignant transformation. Publications resulting from the use of DNA vaccines in clinical studies under IND indicate that intramuscular, subcutaneous, intradermal, or particle-mediated delivery does not result in long-term persistence of plasmid at ectopic sites, and that <30 copies of plasmid per 10^5 host cells persist at the site of injection after 60 days (see Section VI, References). Before conducting biodistribution/persistence studies, you should contact FDA for advice concerning the need for these studies in particular, when: i) new or significantly modified plasmids are proposed for clinical use, and/or ii) the formulation of the DNA vaccine and/or its method/route of delivery may significantly increase cellular uptake or alter plasmid distribution.

We recommend that you conduct biodistribution/persistence studies when modifications to the vector, inserted gene, method of delivery, route of administration, or formulation significantly impact cellular uptake or immunogenicity. We recommend that all preclinical immunogenicity, toxicity and biodistribution/persistence studies evaluate the formulation and method of administration proposed for the clinical study. This would include assessing any adjuvant or active excipient in the vaccine, and/or the use of a device to administer the vaccine. A typical biodistribution study will assess the presence of plasmid collected from a panel of tissues at intervals of 7, 30, and 60 days post-administration. The panel of tissues typically includes the blood, heart, brain, liver, kidney, bone marrow, ovaries/testes, lung, mesenteric lymph nodes, spleen, adrenal gland, muscle at the site of administration and subcutis at the injection site. The presence of the DNA vaccine is typically evaluated using a semi-quantitative real time polymerase chain reaction (Q-PCR) study validated for sensitivity and specificity. We recommend that such assays be able to detect 1 copy of plasmid in DNA from 10^5 host cells.

Comment:

> ***We are not aware of any evidence to support that sequence changes, limited to the inserted gene (or, for that matter, the plasmid backbone) alter the biodistribution of the plasmid. If the plasmid (vector), route of administration, mode of delivery, formulation and dose (similar quantities) are unchanged, and***

if the insert does not have homologous sequences to the human genome, a change to the gene of interest is unlikely to impact integration or biodistribution. We recommend withdrawing the requirement to repeat biodistribution studies when only the gene is changed, particularly if the insert does not have homologous sequences to the human genome.

In this guidance document it is suggested that persistence of plasmid DNA be reported as plasmid copy numbers per 10^5 host cell genomes. Because laboratory measurements are recorded as plasmid copy number per total amount of DNA in the sample, and because the actual quantity of plasmid DNA contributing to the total DNA is relatively minor, we recommend that the denominator be expressed in what is actually measured, namely the quantity of DNA. In this case, the denominator would be reported as total DNA as opposed to host cell genomes as mentioned in this guideline. Plasmid copy numbers would remain as the value reported in the numerator. Expressing copy numbers per total DNA recovered is consistent with the scientific community.

We have determined that integration studies are not necessary when biodistribution/persistence studies demonstrate that plasmid DNA does not persist in any tissue of any animal at levels exceeding 30 copies per 10^5 cellular genomes at 60 days post vaccination. If the DNA plasmid persists at significantly higher copy number at any site in any animal, we recommend that you study whether the DNA has integrated into the genome of the vaccinated animal. Theoretical concerns regarding DNA vaccine integration include the risk of mutagenesis if plasmid insertion reduces the activity of a tumor suppressor or increases the activity of an oncogene. In addition, integration of a DNA vaccine may result in chromosomal instability through the induction of chromosomal breaks or rearrangements. Typically, Q-PCR is used to detect plasmid DNA in genomic DNA preparations. Specifically designed PCR primers may be used to distinguish between integrated and non-integrated plasmids.

Comment:

The technical feasibility for measurement of plasmid copy number (PCN), the appropriate level for risk of integration and the requirement(s) for the need of biodistribution and/or integration studies need further discussion and clarification. The following discussion and approaches are based on both technical feasibility for product testing and on appropriate scientific rationale which afford sufficient scientific support for beginning a Phase 1 clinical program.

<u>Technical Feasibility for the Measurement of PCN</u>

- ***Although in theory PCR can detect one copy of DNA in a sample, the practical Lower Limit of Detection of "state-of-the-art" Real-Time PCR assays is around 10 copies.***
- ***Please consider the following:***

- *Specimens containing less than about 10 target copies are difficult to sample reliably. PCR reactions with low copy numbers contain a variable number of target copies, including no target copies at all.*
- *The sensitivity of PCR-based assays depends in part on the efficiency of amplification; ideally, the target DNA doubles after each amplification cycle.*
- *PCR amplification is not 100% efficient. Real-Time PCR assays typically have Limits of Detection of about 10 target copies and a Limit of Quantitation in the range of 50-100 copies.*

Appropriate level for risk of integration

- *Regarding FDA's proposal that persistence of significantly more than 30 copies per 10^5 cellular genomes should trigger an integration study:*
  - *Host cell DNA recovery is more accurately expressed in terms of µg of total DNA recovered than by cellular genomes or host cell number. The suggested criteria of 30 copies per 10^5 cellular genomes translates to 45 copies per µg of total DNA.*
  - *Assuming a worst-case scenario that each plasmid copy number detected represents a separate mutation event, the proposed criteria of 45 copies/µg corresponds to a mutation rate ~ 3 logs below the reported spontaneous mutation rate for mammalian tissues ($1.5–7.5 \times 10^4$ mutations/µg DNA).*[a, b]
  - *In both mice and rabbits injected with formulated and non-formulated plasmid DNA products that persist with $10^3–10^4$ copies/ µg at 60 days at the injection site, none of the persisting copies are associated with high molecular weight (host cell) DNA.*
- *Based on these data, we propose 10^3 copies of plasmid DNA/µg of total DNA at 60 days as a conservative trigger for conducting an integration study for prophylactic indications. A higher copy number limit would be expected for plasmid DNA products for therapeutic indications.*

The requirement(s) for the need of biodistribution and/or integration studies

- *Biodistribution and integration studies should not be required for plasmid drug products essentially homologous to plasmid drug products that have previously been shown not to associate closely with host cell genomic DNA. We define homologous plasmid drug products as ones having the same plasmid (vector) where the insert does not have homologous sequences to the human genome, and has the same dose, route of administration, and formulation.*
- *Consideration should also be given to waive integration and biodistribution studies for plasmid DNA products shown previously to be safe in human clinical trials when only minor changes to the formulation have been made.*

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[a] Cole J, Stopeck T. Somatic Mutant Frequency, Mutation Rates and Mutational Spectra in the Human Population In Vivo. Mutation Research 1994; 304:33-105.

[b] Parsons B, McKinzie P. Developing Methods of Genetic Analysis to Improve Cancer Risk Assessment. Regulatory Research Pespectives Journal 2001, vol 1, issue 2.

V. CONCLUSION

This document is intended to inform manufacturers/sponsors about current CBER recommendations related to the development of DNA vaccines. We recommend that manufacturers/sponsors of these products concentrate their efforts on the pivotal preclinical safety issues. CBER recommends early consultation to further discuss the issues related to the development of their vaccine.

Comment:

> ***We agree that consultation with CBER often and early facilitates the development of plasmid DNA vaccines. However, we believe it would be beneficial to address the nonbinding nature of advice given early in the development process, as such advice has the potential to be reversed prior to allowance of the IND. A policy or procedure that assures the consistency of advice provided early in the development process, particularly guidance provided during the pre-IND meeting would greatly enhance the ability of industry to efficiently plan and finance product development.***

We suggest the following relevant references be added to the suggested reading list:

1. *Martin, T., Parker, S.E., Hedstrom, R., Le, T., Hoffman, S.L., Norman, J., Hobart, P., and Lew, D., 1999. Plasmid DNA malaria vaccine: The potential for genomic integration after intramuscular injection. Hum. Gene Ther., 10(5): 759-768.*

2. *Parker, S.E., Borellini, F., Wenk, M.L., Hobart, P., Hoffman, S.L., Hedstrom, R., Le, T., and Norman, J.A., 1999. Plasmid DNA malaria vaccine: Tissue distribution and safety studies in mice and rabbits. Hum. Gene Ther., 10(5): 741-758.*

3. *Parker, S.E., Monteith, D., Horton, H., Hof, R., Hernandez, P., Vilalta, A., Hartikka, J., Hobart, P., Bentley, C.E., Chang, A., Hedstrom, R., Rogers, W.O., Kumar, S., Hoffman, S.L., Norman, J.A., 2001. Safety of a GM-CSF adjuvant-plasmid DNA malaria vaccine. Gene Ther. 8: 1011-1023.*

VI. REFERENCES: REGULATIONS AND APPLICABLE GUIDANCE DOCUMENTS, AND RELEVANT PUBLICATIONS

U.S. CODE OF FEDERAL REGULATIONS

21 CFR PART 50 - Protection of Human Subjects
21 CFR PART 56 - Institutional Review Boards
21 CFR PART 58 - Good Laboratory Practice for Nonclinical Laboratory Studies
21 CFR PART 210 - Current Good Manufacturing Practice in Manufacturing, Processing, Packing, or Holding of Drugs; General
21 CFR PART 211 - Current Good Manufacturing Practice for Finished Pharmaceuticals

21 CFR PART 312 - Investigational New Drug Application
21 CFR PART 600 - Biological Products: General
21 CFR PART 601 - Licensing
21 CFR PART 610 - General Biological Products Standards

POINTS TO CONSIDER DOCUMENTS

Points to Consider in the Production and Testing of New Drugs and Biologicals Produced by Recombinant DNA Technology (4/85)
Supplement to the Points to Consider in the Production and Testing of New Drugs and Biologicals Produced by Recombinant DNA Technology: Nucleic Acid Characterization and Genetic Stability (4/92)
Points to Consider in the Characterization of Cell Lines Used to Produce Biologicals (7/93)
Points to Consider in the Manufacture and Testing of Monoclonal Antibody Products for Human Use (2/97)

INTERNATIONAL CONFERENCE ON HARMONIZATION OF TECHNICAL REQUIREMENTS FOR REGISTRATION OF PHARMACEUTICALS FOR HUMAN USE (ICH) DOCUMENTS

ICH; Guideline for Industry: Detection of Toxicity to Reproduction for Medicinal Products (9/94)
ICH; Guideline for Industry: Detection of Toxicity to Reproduction for Medicinal Products: Addendum on Toxicity to Male Fertility (4/96)
ICH; Quality of Biotechnological Products: Stability Testing of Biotechnological/Biological Products (2/04)

FDA GUIDELINES

FDA Guidance Concerning Demonstration of Comparability of Human Biological
Guideline for Submitting Documentation for the Stability of Human Drugs and Biologics (2/87)
Guideline on Sterile Drug Products Produced by Aseptic Processing (6/87); Draft Guidance for Industry: Sterile Drug Products Produced by Aseptic Processing-Current Good Manufacturing Practice (8/03) (This draft guidance when finalized, will replace the 1987 Industry Guideline on Sterile Drug Products Produced by Aseptic Processing.)
Guideline on Validation of the Limulus Amebocyte Lysate Test As An End-Product Endotoxin Test for Human and Animal Parenteral Drugs, Biological Products and Medical Devices (12/87)
Guideline for the Determination of Test Residual Moisture in Dried Biological Products (1/90)
Guideline on the Preparation of Investigational New Drug Products (3/91)
Products, Including Therapeutic Biotechnology-Derived Products (4/96)
Guidance for Industry: Guidance for Human Somatic Cell Therapy and Gene Therapy

PUBLICATIONS RELEVANT TO THE ISSUE OF DNA VACCINE INDUCED NEONATAL TOLERANCE:

Bona C, Radu D, Kodera T. Molecular studies on the iversification of hemagglutinin-specific human neonatal repertoire subsequent to immunization with naked DNA. Vaccine. 2004 Apr 16; 22(13-14): 1624-30.

Bot A, Bona C. Genetic immunization of neonates. Microbes Infect. 2002 Apr; 4(4): 511-20.

Ichino M, Mor G, Conover J, Weiss WR, Takeno M, Ishii KJ, Klinman DM. Factors associated with the development of neonatal tolerance after the administration of a plasmid DNA vaccine. J Immunol. 1999 Apr 1;162(7): 3814-8.

Wang Y, Xiang Z, Pasquini S, Ertl HC. Immune response to neonatal genetic immunization. Virology. 1997 Feb 17; 228(2): 278-84.

Mor G, Yamshchikov G, Sedegah M, Takeno M, Wang R, Houghten RA, Hoffman S, Klinman DM. Induction of neonatal tolerance by plasmid DNA vaccination of mice. J Clin Invest. 1996 Dec 15; 98(12): 2700-5.

PUBLICATIONS RELEVANT TO THE ISSUE OF PLASMID DNA BIODISTRIBUTION AND PERSISTENCE:

Kim BM, Lee DS, Choi JH, Kim CY, Son M, Suh YS, Baek KH, Park KS, Sung YC, Kim WB. In vivo kinetics and biodistribution of a HIV-1 DNA vaccine after administration in mice. Arch Pharm Res. 2003 Jun; 26(6): 493-8

Pilling AM, Harman RM, Jones SA, McCormack NA, Lavender D, Haworth R. The assessment of local tolerance, acute toxicity, and DNA biodistribution following particle-mediated delivery of a DNA vaccine to minipigs. Toxicol Pathol. 2002 May-Jun; 30(3): 298-305.

Bureau MF, Naimi S, Torero Ibad R, Seguin J, Georger C, Arnould E, Maton L, Blanche F, Delaere P, Scherman D. Intramuscular plasmid DNA electrotransfer: biodistribution and degradation. Biochim Biophys Acta. 2004 Jan 20; 1676(2): 138-48.

Wang Z, Troilo PJ, Wang X, Griffiths TG, Pacchione SJ, Barnum AB, Harper LB, Pauley CJ, Niu Z, Denisova L, Follmer TT, Rizzuto G, Ciliberto G, Fattori E, Monica NL, Manam S, Ledwith BJ. Detection of integration of plasmid DNA into host genomic DNA following intramuscular injection and electroporation. Gene Ther. 2004 Apr; 11(8): 711-21.

Ledwith BJ, Manam S, Troilo PJ, Barnum AB, Pauley CJ, Griffiths TG 2nd, Harper LB, Schock HB, Zhang H, Faris JE, Way PA, Beare CM, Bagdon WJ, Nichols WW. Plasmid DNA vaccines: assay for integration into host genomic DNA. Dev Biol (Basel). 2000; 104: 33-43.

Ledwith BJ, Manam S, Troilo PJ, Barnum AB, Pauley CJ, Griffiths TG 2nd, Harper LB, Beare CM, Bagdon WJ, Nichols WW. Plasmid DNA vaccines: investigation of integration into host cellular DNA following intramuscular injection in mice. Intervirology. 2000; 43(4-6): 258-72.

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